

## INVENTION TITLE

CORN PLANT MON88017 AND COMPOSITIONS AND METHODS  
FOR DETECTION THEREOF

## DESCRIPTION

[Para 1] This application claims the benefit of U.S. Provisional Application No. 60/529,477, filed December 15, 2003.

[Para 2] FIELD OF THE INVENTION

[Para 3] The present invention relates to the field of plant molecular biology. More specifically, the invention relates to a glyphosate tolerant and insect resistant corn plant MON88017 and to assays and methods for detecting the presence of corn plant MON88017 DNA in a plant sample and compositions thereof.

[Para 4] DESCRIPTION OF THE RELATED ART

[Para 5] Corn is an important crop and is a primary food source in many areas of the world. The methods of biotechnology have been applied to corn for improvement of the agronomic traits and the quality of the product. One such agronomic trait is herbicide tolerance, in particular, tolerance to glyphosate herbicide. This trait in corn has been conferred by the expression of a transgene in the corn plant that expresses a glyphosate resistant 5-enolpyruvyl-3-phosphoshikimate synthase (CP4 EPSPS, US Patent 5,633,435). Another agronomic trait is insect resistance, for example genetically engineered corn plant resistance to the corn borer and the corn rootworm (US Patent 6,489,542 and US Patent 6,620,988). It would be advantageous to be able to detect the presence of transgene/genomic DNA of a particular plant in order

to determine whether progeny of a sexual cross contain the transgene/genomic DNA of interest. In addition, a method for detecting a particular plant would be helpful when complying with regulations requiring the pre-market approval and labeling of foods derived from the recombinant crop plants.

[Para 6] The expression of foreign genes in plants is known to be influenced by their chromosomal position, perhaps due to chromatin structure (*e.g.*, heterochromatin) or the proximity of transcriptional regulation elements (*e.g.*, enhancers) close to the integration site (Weising *et al.*, Ann. Rev. Genet 22:421–477, 1988). For this reason, it is often necessary to screen a large number of plants in order to identify a plant characterized by optimal expression of an introduced gene of interest. For example, it has been observed in plants and in other organisms that there may be a wide variation in levels of expression of an introduced transgene among plants. There may also be differences in spatial or temporal patterns of expression, for example, differences in the relative expression of a transgene in various plant tissues, that may not correspond to the patterns expected from transcriptional regulatory elements present in the introduced gene construct. For this reason, it is common to produce hundreds to thousands of different transgenic plants and screen those plants for a single plant that has desired transgene expression levels and phenotype for commercial purposes. A plant that has desired levels or patterns of transgene expression is useful for introgressing the transgene into other genetic backgrounds by sexual crossing using conventional breeding methods. Progeny of such crosses maintain the transgene expression characteristics of the original transformant. This strategy is used to ensure reliable gene expression in a number of varieties that are well adapted to local growing conditions and market demands.

[Para 7] It is possible to detect the presence of a transgene by any well-known nucleic acid detection methods such as the polymerase chain reaction (PCR) or DNA hybridization using polynucleic acid probes. These detection methods generally use DNA primer or probe molecules that are specific to the genetic elements, such as promoters, leaders, introns, coding regions, 3' transcription terminators, marker genes, *etc*, that are the components of the transgenes of a DNA construct. As a result, such methods may not be useful for discriminating between different transgenic events, particularly those produced using the same transgene DNA construct unless the sequence of genomic DNA adjacent to the inserted transgene DNA is known. Event-specific DNA detection methods have been developed for many transgenic crop plant introductions, for example sugar beet (US patent 6,531,649), wheat (US patent pub 20020062503), insect resistant corn (US patent pub 20020102582), and a glyphosate tolerant corn event nk603 (US patent pub 20020013960).

[Para 8] The present invention relates to a glyphosate tolerant and corn rootworm resistant corn plant MON88017, and compositions contained therein, and to the method for the detection of the transgene/genomic insertion region in corn plant MON88017 and progeny thereof containing these compositions.

#### [Para 9] SUMMARY OF THE INVENTION

[Para 10] The present invention is related to the transgenic corn plant designated MON88017 having seed deposited with American Type Culture Collection (ATCC) with Accession No. PTA-5582. Another aspect of the invention comprises the progeny plants, or seeds, or regenerable parts of the plants and seeds of the plant MON88017. The invention also includes plant parts of corn plant MON88017 that include, but are not limited to pollen, ovule, seed, roots, and leaves. The invention relates to a corn plant MON88017 having a glyphosate tolerant phenotype and a

corn rootworm resistant phenotype and the novel genetic compositions contained in the genome of MON88017.

[Para 11] One aspect of the invention provides DNA compositions and methods for detecting the presence of a transgene/genomic junction region from corn plant MON88017. Isolated DNA molecules are provided that comprise at least one transgene/genomic junction DNA molecule selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2, and complements thereof, wherein the junction molecule spans the transgene insertion site, which comprises a heterologous DNA inserted into the corn genome and corn genomic DNA flanking the insertion site in corn plant MON88017. A corn seed and plant material thereof comprising any one of these DNA molecules is an aspect of this invention.

[Para 12] An isolated DNA molecule is provided that is a transgene/genomic region SEQ ID NO:3 or the complement thereof, wherein this DNA molecule is novel in the genome of corn plant MON88017. A corn plant and seed comprising SEQ ID NO:3 in its genome is an aspect of this invention.

[Para 13] According to another aspect of the invention, an isolated DNA molecule is provided that is a transgene/genomic region SEQ ID NO:4, or the complement thereof, wherein this DNA molecule is novel in the genome of corn plant MON88017. A corn plant and seed comprising SEQ ID NO:4 in its genome is an aspect of this invention.

[Para 14] An isolated DNA molecule is provided that is a transgene/genomic region, SEQ ID NO:5 of MON88017 or the complement thereof, wherein this DNA molecule is novel in the genome of corn plant MON88017. A corn plant and seed comprising SEQ ID NO:5 in its genome is an aspect of this invention.

[Para 15] According to another aspect of the invention, two DNA molecules comprising a primer pair are provided for use in a DNA

detection method, wherein the first DNA molecule comprises at least 11 or more contiguous polynucleotides of any portion of the transgene DNA region of the DNA molecule of SEQ ID NO:3 or the complement thereof, and a second DNA molecule comprising at least 11 or more contiguous polynucleotides of any portion of a corn genomic DNA region of SEQ ID NO:3 or complement thereof, wherein these DNA molecules when used together comprise a DNA primer set in a DNA amplification method that produces an amplicon. The amplicon produced using the DNA primer pair in the DNA amplification method is diagnostic for corn plant MON88017 when the amplicon contains SEQ ID NO:1. Any length amplicon produced from MON88017 DNA wherein the amplicon comprises SEQ ID NO:1 is an aspect of the invention. The skilled artisan will recognize that the first and second DNA molecules are not required to consist only of DNA but may also be comprised exclusively of RNA, a mixture of DNA and RNA, or a combination of DNA, RNA, or other nucleotides or analogues thereof that do not act as templates for one or more polymerases. In addition, the skilled artisan will recognize that a probe or a primer as set forth herein shall be at least from about 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 consecutive nucleotides in length and selected from the group of nucleotides as set forth in SEQ ID NO:1 (arbitrarily designated 5' junction), SEQ ID NO:2 (arbitrarily designated 3' junction), SEQ ID NO:3 (portion of the arbitrarily designated 5' flanking sequence), SEQ ID NO:4 (portion of the arbitrarily designated 3' flanking sequence), and SEQ ID NO:5 (all or a portion of the inserted nucleotide sequence). Probes and primers at least from about 21 to about 50 or more consecutive nucleotides in length are possible when selected from the group of nucleotides as set forth in SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.

[Para 16] According to another aspect of the invention, two DNA molecules comprising a primer pair are provided for use in a DNA detection method, wherein the first DNA molecule comprises at

least 11 or more contiguous polynucleotides of any portion of the transgene DNA region of the DNA molecule of SEQ ID NO:4 or the complement thereof, and a second DNA molecule comprising at least 11 or more contiguous polynucleotides of any portion of a corn genomic DNA region of SEQ ID NO:4 or complement thereof, wherein these DNA molecules when used together comprise a DNA primer set in a DNA amplification method that produces an amplicon. The amplicon produced using the DNA primer pair in the DNA amplification method is diagnostic for corn plant MON88017 when it comprises SEQ ID NO:2. Any length amplicon produced from MON88017 DNA, wherein the amplicon comprises SEQ ID NO:2 is an aspect of the invention.

[Para 17] According to another aspect of the invention, methods of detecting the presence of DNA corresponding specifically to the corn plant MON88017 DNA in a sample are provided. Such methods comprise: (a) contacting the sample comprising MON88017 genomic DNA with a DNA primer pair; and (b) performing a nucleic acid amplification reaction, thereby producing an amplicon; and (c) detecting the amplicon, wherein the amplicon comprises SEQ ID NO:1 or SEQ ID NO:2.

[Para 18] According to another aspect of the invention, methods of detecting the presence of DNA corresponding specifically to the corn plant MON88017 DNA in a sample are provided. Such methods comprising: (a) contacting the sample comprising MON88017 DNA with a DNA probe comprising SEQ ID NO:1 or SEQ ID NO:2, or DNA molecules substantially homologous to SEQ ID NO:1 or SEQ ID NO:2 that hybridize under stringent hybridization conditions with genomic DNA from corn plant MON88017 and does not hybridize under the stringent hybridization conditions with non-MON88017 corn plant DNA; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the corn plant MON88017 DNA.

[Para 19] According to another aspect of the invention, methods of producing a corn plant that tolerates application of glyphosate and are resistant to corn rootworm are provided that comprise the step of: sexually crossing a first parental corn plant MON88017 with a second parental corn plant that lacks the glyphosate tolerance and corn rootworm resistance, thereby producing hybrid progeny plants that are glyphosate tolerant and corn rootworm resistant.

[Para 20] In another aspect of the invention is a method of determining the zygosity of the progeny of corn event MON88017 comprising: (a) contacting the sample comprising corn DNA with a primer set comprising SEQ ID NO:32, SEQ ID NO:33 and SEQ ID NO:34, that when used in a nucleic-acid amplification reaction with genomic DNA from corn event MON88017, produces a first amplicon that is diagnostic for corn event MON88017; and (b) performing a nucleic acid amplification reaction, thereby producing the first amplicon; and (c) detecting the first amplicon; and (d) contacting the sample comprising corn DNA with said primer set, that when used in a nucleic-acid amplification reaction with genomic DNA from corn plants produces a second amplicon comprising the native corn genomic DNA homologous to the corn genomic region of a transgene insertion identified as corn event MON88017; and (e) performing a nucleic acid amplification reaction, thereby producing the second amplicon; and (f) detecting the second amplicon; and (g) comparing the first and second amplicons in a sample, wherein the presence of both amplicons indicates the sample is heterozygous for the transgene insertion.

[Para 21] A method for controlling weeds in a field of corn plant MON88017 comprising the step of applying an effective amount of glyphosate containing herbicide to the field of MON88017 corn plants.

[Para 22] A hybrid corn seed comprising wherein at least one parent is MON88017.

[Para 23] A corn plant transformed with a plant DNA construct comprising the plant expression cassettes of pMON53616.

[Para 24] The foregoing and other aspects of the invention will become more apparent from the following detailed description and accompanying drawings.

[Para 25] BRIEF DESCRIPTION OF THE DRAWINGS

[Para 26] Figure 1. Plasmid map of pMON53616.

[Para 27] Figure 2. Genomic organization of insert in corn plant MON88017.

[Para 28] Figure 3. MON88017 5' transgene/genomic DNA region (SEQ ID NO:3).

[Para 29] Figure 4. MON88017 3' transgene/genomic DNA region (SEQ ID NO:4).

[Para 30] Figure 5. MON88017 transgene/genomic DNA region (SEQ ID NO:5)

[Para 31] DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[Para 32] A transgenic corn plant, herein referred to as "plant MON88017" or "MON88017", is resistant to feeding damage by the Coleopteran pest corn rootworm (*Diabrotica* spp.), and is tolerant to the phytotoxic action of glyphosate containing agricultural herbicides. This dual-trait corn plant expresses a modified variant of the CRY3Bb1 protein (US Patent 6,501,009) from *Bacillus thuringiensis* (subsp. *kumamotoensis*), that provides resistance to feeding damage by corn rootworm larvae, and a glyphosate resistant 5-enolpyruvylshikimate-3-phosphate



synthase (CP4 EPSPS) protein (US Patent 5,633,435) from *Agrobacterium* sp. strain CP4 that confers plant tolerance to glyphosate. Use of the dual-trait corn will provide major benefits to corn growers: a) protection from economic losses due to the corn rootworm larvae, a major insect pest in the U.S. and growing concern in many corn growing areas of the world, and b) the ability to apply glyphosate containing agricultural herbicides to the corn crop for broad-spectrum weed control. Additionally, the transgenes encoding the corn rootworm and glyphosate tolerant traits are linked on the same DNA segment and occur at a single locus in the genome of MON88017, this provides for enhanced breeding efficiency and enables the use of molecular markers to track the transgene insert in the breeding populations and progeny thereof.

[Para 33] The corn plant MON88017 was produced by an *Agrobacterium* mediated transformation process of an inbred corn line with the plasmid construct pMON53616 (Figure 1). This plasmid construct contains the linked plant expression cassettes with the regulatory genetic elements necessary for expression of the CRY3Bb1 protein and the CP4 EPSPS protein in corn plant cells. Corn cells were regenerated into intact corn plants and individual plants were selected from the population of plants that showed integrity of the plant expression cassettes and resistance to glyphosate and corn rootworm larvae feeding damage. A corn plant that contains in its genome the linked plant expression cassettes of pMON53616 is an aspect of the present invention.

[Para 34] The plasmid DNA inserted into the genome of corn plant MON88017 was characterized by detailed molecular analyses. These analyses included: the insert number (number of integration sites within the corn genome), the copy number (the number of copies of the T-DNA within one locus), and the integrity of the inserted gene cassettes. DNA molecular probes were used that included the intact CP4 EPSPS and CRY3Bb1 coding

regions and their respective regulatory elements, the promoters, introns, and polyadenylation sequences of the plant expression cassettes, and the plasmid pMON53616 backbone DNA region. The data show that MON88017 contains a single T-DNA insertion with one copy of both the CRY3Bb1 and the CP4 EPSPS cassettes. No additional elements from the transformation vector pMON53616, linked or unlinked to intact gene cassettes, were detected in the genome of MON88017. Finally, PCR and DNA sequence analyses were performed to determine the 5' and 3' insert-to-plant genome junctions, confirm the organization of the elements within the insert (Figure 2), and determine the complete DNA sequence of the insert in corn plant MON88017 (SEQ ID NO:5).

[Para 35] A glyphosate tolerant, corn rootworm resistant corn plant can be bred by first sexually crossing a first parental corn plant, consisting of a corn plant grown from MON88017, with a second parental corn plant that lacks the tolerance to glyphosate herbicide, thereby producing a plurality of hybrid progeny plants. Inbred corn lines can be generated by a process that includes, backcrossing with the recurrent parent, and selection with glyphosate treatment. Descriptions of other breeding methods that are commonly used for different traits and crops can be found in references known in the art, *e.g.*, Fehr, in *Breeding Methods for Cultivar Development*, Wilcox J. ed., American Society of Agronomy, Madison WI (1987).

[Para 36] Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Definitions of common terms in molecular biology may also be found in Rieger *et al.*, *Glossary of Genetics: Classical and Molecular*, 5th edition, Springer-Verlag: New York, 1991; and Lewin, *Genes V*, Oxford University Press: New York, 1994. The nomenclature for DNA bases as set forth at 37 CFR § 1.822 is used.

[Para 37] As used herein, the term “corn” means *Zea mays* and includes all plant varieties that can be bred with corn plant MON88017.

[Para 38] As used herein, the term “comprising” means “including but not limited to”.

[Para 39] “Glyphosate” refers to N-phosphonomethylglycine and its salts. N-phosphonomethylglycine is a well-known herbicide that has activity on a broad spectrum of plant species. Glyphosate is the active ingredient of Roundup® (Monsanto Co.), a safe herbicide having a desirably short half-life in the environment. Glyphosate is the active ingredient of Roundup® herbicide (Monsanto Co.). Treatments with “glyphosate herbicide” refer to treatments with the Roundup®, Roundup Ultra®, Roundup Pro® herbicide or any other herbicide formulation containing glyphosate. Examples of commercial formulations of glyphosate include, without restriction, those sold by Monsanto Company as ROUNDUP®, ROUNDUP® ULTRA, ROUNDUP® ULTRAMAX, ROUNDUP® WEATHERMAX, ROUNDUP® CT, ROUNDUP® EXTRA, ROUNDUP® BIACTIVE, ROUNDUP® BIOFORCE, RODEO®, POLARIS®, SPARK® and ACCORD® herbicides, all of which contain glyphosate as its isopropylammonium salt; those sold by Monsanto Company as ROUNDUP® DRY and RIVAL® herbicides, which contain glyphosate as its ammonium salt; that sold by Monsanto Company as ROUNDUP® GEOFORCE, which contains glyphosate as its sodium salt; and that sold by Syngenta Crop Protection as TOUCHDOWN® herbicide, which contains glyphosate as its trimethylsulfonium salt. When applied to a plant surface, glyphosate moves systemically through the plant. Glyphosate is phytotoxic due to its inhibition of the shikimic acid pathway, which provides a precursor for the synthesis of aromatic amino acids. Glyphosate inhibits the enzyme 5-enolpyruvyl-3-phosphoshikimate synthase (EPSPS) found in plants. Glyphosate tolerance can be achieved by the expression of bacterial EPSPS

variants and plant EPSPS variants that have lower affinity for glyphosate and therefore retain their catalytic activity in the presence of glyphosate (U.S. Patent Nos. 5,633,435, 5,094,945, 4,535,060, and 6,040,497).

[Para 40] Corn rootworm (CRW, *Diabrotica spp*) larvae feed on the roots of developing corn plants. Substantial expense and time is devoted to controlling the economic damage caused by this pest. Chemical insecticides, including organophosphates, carbamates and pyrethroids are incorporated into the soil on over 16 million corn acres annually to control CRW. The benefits of shifting away from soil insecticides to a transgenic approach are impressive and include a reduction in potential human health and safety risks, reduced direct impacts on nontarget organisms, reduced contamination of surface and ground water supplies, decreased pesticide container disposal problems, and general compatibility with other pest management and agronomic programs.

[Para 41] The present invention provides for transgenic plants which have been transformed with a DNA construct that contains at least one expression cassette that expresses high levels of CRY3Bb1 delta-endotoxin and at least one expression cassette that expresses a glyphosate tolerance enzyme. Corn plants transformed according to the methods and with the DNA construct disclosed herein are resistant to CRW. The same corn plants are also resistant to glyphosate herbicide. The linked agronomic traits provide ease in maintaining these traits together in a breeding population.

[Para 42] A transgenic "plant" is produced by transformation of a plant cell with heterologous DNA, *i.e.*, a polynucleic acid construct that includes a transgene of interest; regeneration of a population of plants resulting from the insertion of the transgene into the genome of the plant cell, and selection of a particular plant characterized by insertion into a particular genome location. The

term "event" refers to the original transformant plant and progeny of the transformant that include the heterologous DNA. The term "event" also includes progeny produced by a sexual outcross between the event and another plant wherein the progeny includes the heterologous DNA. Even after repeated back-crossing to a recurrent parent, the inserted DNA and flanking genomic DNA from the transformed parent event is present in the progeny of the cross at the same chromosomal location. The term "event" also refers to DNA from the original transformant comprising the inserted DNA, and flanking genomic sequence immediately adjacent to the inserted DNA, that would be expected to be transferred to a progeny that receives the inserted DNA including the transgene of interest as the result of a sexual cross of one parental line that includes the inserted DNA (*e.g.*, the original transformant and progeny resulting from selfing) and a parental line that does not contain the inserted DNA. The present invention is related to the transgenic event, corn plant MON88017, progeny thereof, and DNA compositions contained therein.

[Para 43] A "probe" is an isolated nucleic acid to which is attached a conventional detectable label or reporter molecule, *e.g.*, a radioactive isotope, ligand, chemiluminescent agent, or enzyme. Such a probe is complementary to a strand of a target nucleic acid, in the case of the present invention, to a strand of genomic DNA from MON88017 whether from a MON88017 plant or from a sample that includes MON88017 DNA. Probes according to the present invention include not only deoxyribonucleic or ribonucleic acids, but also polyamides and other probe materials that bind specifically to a target DNA sequence and can be used to detect the presence of that target DNA sequence.

[Para 44] DNA primers are isolated polynucleic acids that are annealed to a complementary target DNA strand by nucleic acid

hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, *e.g.*, a DNA polymerase. A DNA primer pair or a DNA primer set of the present invention refer to two DNA primers useful for amplification of a target nucleic acid sequence, *e.g.*, by the polymerase chain reaction (PCR) or other conventional polynucleic acid amplification methods.

[Para 45] DNA probes and DNA primers are generally 11 polynucleotides or more in length, often 18 polynucleotides or more, 24 polynucleotides or more, or 30 polynucleotides or more. Such probes and primers are selected to be of sufficient length to hybridize specifically to a target sequence under high stringency hybridization conditions. Preferably, probes and primers according to the present invention have complete sequence similarity with the target sequence, although probes differing from the target sequence that retain the ability to hybridize to target sequences may be designed by conventional methods.

[Para 46] Methods for preparing and using probes and primers are described, for example, in *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989 (hereinafter, "Sambrook *et al.*, 1989"); *Current Protocols in Molecular Biology*, ed. Ausubel *et al.*, Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel *et al.*, 1992"); and Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press: San Diego, 1990. PCR DNA primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

[Para 47] Primers and probes based on the flanking genomic DNA and insert sequences disclosed herein can be used to confirm (and, if necessary, to correct) the disclosed DNA

sequences by conventional methods, *e.g.*, by re-cloning and sequencing such DNA molecules.

[Para 48] The nucleic acid probes and primers of the present invention hybridize under stringent conditions to a target DNA molecule. Any conventional nucleic acid hybridization or amplification method can be used to identify the presence of DNA from a transgenic plant in a sample. Polynucleic acid molecules, also referred to as nucleic acid segments, or fragments thereof are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two polynucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, 1989, and by Haymes *et al.*, In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985), Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. In order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form

a stable double-stranded structure under the particular solvent and salt concentrations employed.

[Para 49] As used herein, a substantially homologous sequence is a nucleic acid sequence that will specifically hybridize to the complement of the nucleic acid sequence to which it is being compared under high stringency conditions. Appropriate stringency conditions that promote DNA hybridization, for example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1–6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed. In a preferred embodiment, a polynucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NOs: 1, 2, 3, 4, or 5, or complements thereof or fragments of either under moderately stringent conditions, for example at about 2.0 x SSC and about 65°C. In a particularly preferred embodiment, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NOs: 1, 2, 3, 4, or 5 or complements or fragments of either under high stringency conditions. In one aspect of the present invention, a preferred marker nucleic acid molecule of the present invention has the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or complements thereof or fragments of either. In another aspect of the present invention, a preferred marker nucleic acid molecule of the present invention shares between 80% and 100% or 90% and



100% sequence identity with the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or complement thereof or fragments of either. In a further aspect of the present invention, a preferred marker nucleic acid molecule of the present invention shares between 95% and 100% sequence identity with the sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or complement thereof or fragments of either. SEQ ID NO:1 or SEQ ID NO:2 may be used as markers in plant breeding methods to identify the progeny of genetic crosses similar to the methods described for simple sequence repeat DNA marker analysis, in "DNA markers: Protocols, applications, and overviews: (1997) 173–185, Cregan, *et al.*, eds., Wiley-Liss NY. The hybridization of the probe to the target DNA molecule can be detected by any number of methods known to those skilled in the art, these can include, but are not limited to, fluorescent tags, radioactive tags, antibody based tags, and chemiluminescent tags.

[Para 50] Regarding the amplification of a target nucleic acid sequence (*e.g.*, by PCR) using a particular amplification primer pair, "stringent conditions" are conditions that permit the primer pair to hybridize only to the target nucleic acid sequence to which a primer having the corresponding wild-type sequence (or its complement) would bind and preferably to produce a unique amplification product, the amplicon, in a DNA thermal amplification reaction.

[Para 51] The term "specific for (a target sequence)" indicates that a probe or primer hybridizes under stringent hybridization conditions only to the target sequence in a sample comprising the target sequence.

[Para 52] As used herein, "amplified DNA" or "amplicon" refers to the product of polynucleic acid amplification method directed to a target polynucleic acid molecule that is part of a polynucleic acid template. For example, to determine whether a corn plant resulting from a sexual cross contains transgenic plant genomic

DNA from the corn plant MON88017 plant of the present invention, DNA that is extracted from a corn plant tissue sample may be subjected to a polynucleic acid amplification method using a primer pair that includes a primer derived from DNA sequence in the genome of the MON88017 plant adjacent to the insertion site of the inserted heterologous DNA (transgene DNA), and a second primer derived from the inserted heterologous DNA to produce an amplicon that is diagnostic for the presence of the MON88017 plant DNA. The diagnostic amplicon is of a length and has a DNA sequence that is also diagnostic for the plant genomic DNA, the DNA sequence of the amplicon comprising SEQ ID NO:1 or SEQ ID NO:2. The amplicon may range in length from the combined length of the primer pair plus one nucleotide base pair, preferably plus about fifty nucleotide base pairs, more preferably plus about two hundred-fifty nucleotide base pairs, and even more preferably plus about four hundred-fifty nucleotide base pairs or more. Alternatively, a primer pair can be derived from genomic sequence on both sides of the inserted heterologous DNA so as to produce an amplicon that includes the entire insert polynucleotide sequence (*e.g.*, a forward primer isolated from the genomic portion of SEQ ID NO:3 and a reverse primer isolated from the genomic portion of SEQ ID NO:4 that amplifies a DNA molecule comprising the two expression cassettes of pMON53616 DNA fragment that was inserted into the MON88017 genome, the insert comprising about 7125 nucleotides of SEQ ID NO:5, Figure 2 and Figure 5). A member of a primer pair derived from the plant genomic sequence adjacent to the transgene insert DNA is located a distance from the inserted DNA sequence, this distance can range from one nucleotide base pair up to about twenty thousand nucleotide base pairs. The use of the term "amplicon" specifically excludes primer dimers that may be formed in the DNA thermal amplification reaction.

[Para 53] Polynucleic acid amplification can be accomplished by any of the various polynucleic acid amplification methods known in the art, including the polymerase chain reaction (PCR). Amplification methods are known in the art and are described, *inter alia*, in U.S. Patent Nos. 4,683,195 and 4,683,202 and in *PCR Protocols: A Guide to Methods and Applications*, ed. Innis *et al.*, Academic Press, San Diego, 1990. PCR amplification methods have been developed to amplify up to 22 kb (kilobase) of genomic DNA and up to 42 kb of bacteriophage DNA (Cheng *et al.*, Proc. Natl. Acad. Sci. USA 91:5695–5699, 1994). These methods as well as other methods known in the art of DNA amplification may be used in the practice of the present invention. The sequence of the heterologous DNA insert or flanking genomic DNA sequence from MON88017 can be verified (and corrected if necessary) by amplifying such DNA molecules from the MON88017 seed or plants grown from the seed deposited with the ATCC having accession no. PTA-5582, using primers derived from the sequences provided herein, followed by standard DNA sequencing of the PCR amplicon or cloned DNA fragments thereof.

[Para 54] DNA detection kits that are based on DNA amplification methods contain DNA primer molecules that hybridize specifically to a target DNA and amplify a diagnostic amplicon under the appropriate reaction conditions. The kit may provide an agarose gel based detection method or any number of methods of detecting the diagnostic amplicon that are known in the art. A kit that contains DNA primers that are homologous or complementary to any portion of the corn genomic region of SEQ ID NO:3 or SEQ ID NO:4 and to any portion of the transgene insert region of SEQ ID NO:5 is an object of the invention. Specifically identified as a useful primer pair in a DNA amplification method is SEQ ID NO:6 and SEQ ID NO:7 that amplify a diagnostic amplicon homologous to a portion of the 5' transgene/genome region of MON88017, wherein the amplicon comprises SEQ ID NO:1. Other DNA molecules useful as DNA primers can be

selected from the disclosed transgene/genomic DNA sequence of MON88017 (SEQ ID NO:5) by those skilled in the art of DNA amplification.

[Para 55] The diagnostic amplicon produced by these methods may be detected by a plurality of techniques. One such method is Genetic Bit Analysis (Nikiforov, *et al.* Nucleic Acid Res. 22:4167-4175, 1994) where a DNA oligonucleotide is designed that overlaps both the adjacent flanking genomic DNA sequence and the inserted DNA sequence. The oligonucleotide is immobilized in wells of a microtiter plate. Following PCR of the region of interest (using one primer in the inserted sequence and one in the adjacent flanking genomic sequence), a single-stranded PCR product can be hybridized to the immobilized oligonucleotide and serve as a template for a single base extension reaction using a DNA polymerase and labelled dideoxynucleotide triphosphates (ddNTPs) specific for the expected next base. Readout may be fluorescent or ELISA-based. A signal indicates presence of the transgene/genomic sequence due to successful amplification, hybridization, and single base extension.

[Para 56] Another method is the Pyrosequencing technique as described by Winge (Innov. Pharma. Tech. 00:18-24, 2000). In this method an oligonucleotide is designed that overlaps the adjacent genomic DNA and insert DNA junction. The oligonucleotide is hybridized to single-stranded PCR product from the region of interest (one primer in the inserted sequence and one in the flanking genomic sequence) and incubated in the presence of a DNA polymerase, ATP, sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate and luciferin. DNTPs are added individually and the incorporation results in a light signal that is measured. A light signal indicates the presence of the transgene/genomic sequence due to successful amplification, hybridization, and single or multi-base extension.

[Para 57] Fluorescence Polarization as described by Chen, *et al.*, (Genome Res. 9:492–498, 1999) is a method that can be used to detect the amplicon of the present invention. Using this method an oligonucleotide is designed that overlaps the genomic flanking and inserted DNA junction. The oligonucleotide is hybridized to single-stranded PCR product from the region of interest (one primer in the inserted DNA and one in the flanking genomic DNA sequence) and incubated in the presence of a DNA polymerase and a fluorescent-labeled ddNTP. Single base extension results in incorporation of the ddNTP. Incorporation can be measured as a change in polarization using a fluorometer. A change in polarization indicates the presence of the transgene/genomic sequence due to successful amplification, hybridization, and single base extension.

[Para 58] Taqman® (PE Applied Biosystems, Foster City, CA) is described as a method of detecting and quantifying the presence of a DNA sequence and is fully understood in the instructions provided by the manufacturer. Briefly, a FRET oligonucleotide probe is designed that overlaps the genomic flanking and insert DNA junction. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking genomic sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Hybridization of the FRET probe results in cleavage and release of the fluorescent moiety away from the quenching moiety on the FRET probe. A fluorescent signal indicates the presence of the transgene/genomic sequence due to successful amplification and hybridization.

[Para 59] Molecular Beacons have been described for use in sequence detection as described in Tyangi, *et al.* (Nature Biotech. 14:303–308, 1996) Briefly, a FRET oligonucleotide probe is designed that overlaps the flanking genomic and insert DNA junction. The unique structure of the FRET probe results in it containing secondary structure that keeps the fluorescent and

quenching moieties in close proximity. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking genomic sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Following successful PCR amplification, hybridization of the FRET probe to the target sequence results in the removal of the probe secondary structure and spatial separation of the fluorescent and quenching moieties. A fluorescent signal results. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

[Para 60] DNA detection kits can be developed using the compositions disclosed herein and the methods well known in the art of DNA detection. The kits are useful for identification of corn plant MON88017 DNA in a sample and can be applied to methods for breeding corn plants containing MON88017 DNA. A kit contains DNA molecules that are useful as primers or probes and that are homologous or complementary to at least a portion of SEQ ID NO:1, 2, 3, 4, or 5. The DNA molecules can be used in DNA amplification methods (PCR) or as probes in polynucleic acid hybridization methods, *i.e.*, Southern analysis, northern analysis. The plasmid vector pMON53616 used to produce corn event MON88017 contains two expression cassettes: expression cassette one comprises the CaMV 35S promoter with duplicated enhancer linked to the wheat CAB 5' leader linked to the rice actin 1 intron linked to the CRY3Bb1 coding region linked to the wheat Hsp17 3' polyadenylation sequence; and linked to expression cassette two that comprises the rice actin 1 promoter linked to the rice actin 1 intron driving transcription of a chloroplast transit peptide fused to the CP4 EPSPS coding region and linked to the NOS 3' polyadenylation region, the sequence of the linked expression cassettes is contained in SEQ ID NO:5.

[Para 61] The following examples are included to demonstrate examples of certain preferred embodiments of the invention. It

should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent approaches the inventors have found function well in the practice of the invention, and thus can be considered to constitute examples of preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[Para 62] EXAMPLES

[Para 63] EXAMPLE 1

[Para 64] The transgenic corn plant MON88017 was generated by an *Agrobacterium*-mediated transformation of corn cells with a DNA fragment derived from pMON53616 (Figure 1). The plant transformation construct, pMON53616 was mated into *Agrobacterium* using a triparental mating procedure (Ditta *et al.*, Proc. Natl. Acad. Sci. 77:7347-7351, 1980). The DNA fragment of pMON53616 that contains two transgene plant expression cassettes was inserted into the genome of a corn plant cell, the corn plant cell was regenerated into corn plant MON88017. The configuration of the insert into the genome of MON88017 is shown in (Figure 2). MON88017 and its progeny have tolerance to glyphosate and are resistant to corn rootworm larvae feeding damage. Corn transformation was performed essentially as described herein.

[Para 65] Liquid cultures of *Agrobacterium* containing pMON53616 are initiated from glycerol stocks or from a freshly streaked plate and grown overnight at 26°C-28°C with shaking (approximately 150 revolutions per minute, rpm) to mid-log growth phase in liquid LB medium, pH 7.0, containing 50 mg/l

(milligram per liter) kanamycin, and either 50 mg/l streptomycin or 50 mg/l spectinomycin, and 25 mg/l chloramphenicol with 200  $\mu$ M acetosyringone (AS). The *Agrobacterium* cells are resuspended in the inoculation medium (liquid CM4C, as described in Table 8 of U.S. Patent 6,573,361) and the cell density is adjusted such that the resuspended cells have an optical density of 1 when measured in a spectrophotometer at a wavelength of 660 nm (*i.e.*, OD<sub>660</sub>). Freshly isolated Type II immature HillxLH198 and Hill corn embryos are inoculated with *Agrobacterium* and co-cultured 2–3 days in the dark at 23°C. The embryos are then transferred to delay media (N6 1–100–12; as described in Table 1 of U.S. Patent 5,424,412) supplemented with 500 mg/l Carbenicillin (Sigma–Aldrich, St Louis, MO) and 20  $\mu$ M AgNO<sub>3</sub>) and incubated at 28 °C for 4 to 5 days. All subsequent cultures are kept at this temperature.

[Para 66] The corn coleoptiles are removed one week after inoculation. The embryos are transferred to the first selection medium (N61–0–12, as described in Table 1 of U.S. Patent 5,424,412), supplemented with 500 mg/l Carbenicillin and 0.5 mM glyphosate. Two weeks later, surviving tissues are transferred to the second selection medium (N61–0–12) supplemented with 500mg/l Carbenicillin and 1.0 mM glyphosate. Surviving callus is subcultured every 2 weeks for about 3 subcultures on 1.0 mM glyphosate. When glyphosate tolerant tissues are identified, the tissue is bulked up for regeneration. For regeneration, callus tissues are transferred to the regeneration medium (MSOD, as described in Table 1 of U.S. Patent 5,424,412) supplemented with 0.1  $\mu$ M abscisic acid (ABA; Sigma–Aldrich, St Louis, MO) and incubated for two weeks. The regenerating calli are transferred to a high sucrose medium and incubated for two weeks. The plantlets are transferred to MSOD media (without ABA) in a culture vessel and incubated for two weeks. Then the plants with roots are transferred into soil. Those skilled in the art of corn cell transformation methods can



modify this method to provide transgenic corn plants containing the DNA construct of the present invention, or use other methods, such as, particle gun, that are known to provide transgenic monocot plants.

[Para 67] The MON88017 plant and seed has regenerable parts. The regenerable parts of the seed include, but are not limited to the embryo, the cotyledon, and the shoot or root meristem. The invention also includes plant parts of corn plant MON88017 that include, but are not limited to pollen, ovule, shoots, roots, and leaves. The invention also includes extractable components of MON88017 seed that include, but are not limited to protein, meal, flour, and oil.

[Para 68] EXAMPLE 2

[Para 69] The corn plant MON88017 was selected from many transgenic corn plants for tolerance to glyphosate vegetative and reproductive injury. The successful production of a commercial quality transgenic plant currently requires producing a large number of transgenic plants. In the present invention, MON88017 was one plant among approximately 472  $R_0$  events that had been transformed with different DNA constructs that included pMON53616. The MON88017 event was selected from the many events by a series of molecular analysis, glyphosate tolerance screens, insect resistance screens, and expression analysis screens.

[Para 70] Transgenic corn plants were assayed for corn rootworm resistance to feeding damage by greenhouse and field screens. Root damage was rated using the Node-Injury Scale (NIS) developed by Oleson and Tollefson of Iowa State University, this scale rates damage done to corn roots using 0–3 values. The 0.00 value describes no feeding damage and is the lowest rating, 1.00 describes one node or the equivalent of an entire node eaten

back to within approximately two inches of the stalk, 2.00 describes two complete nodes eaten, and 3.00 describes three or more nodes eaten and is the highest rating. Damage in between complete nodes eaten is noted as the percentage of the node missing, i.e., 1.50 describes 1 ½ nodes eaten, 0.25 describes ¼ of one node eaten. Field plots were artificially infested with corn rootworm larvae by applying 1500–2000 eggs per linear foot of row at the V3–V4 growth stage. Insecticide treated rows were treated with Tefluthrin insecticide (Force® 3G, Zeneca Ag Products) at a rate of 5 ounces per 1000 feet of row. MON88017 demonstrated a root damage rating (RDR) of between 0.08 and 0.11 in multiple tests. The non-insecticide treated isolate showed an average RDR of 1.28. Insecticide treated non-insecticidal protein containing corn plants showed an average RDR of 0.44.

[Para 71] The transgenic corn plants were treated with glyphosate herbicide to determine the level of tolerance to the herbicide. Test plots were sprayed with glyphosate (Roundup® WeatherMax, Monsanto Co, St Louis, MO) that was applied twice during the growing season once at the V4 and once at the V8 growth stage at a rate of 1.125 and 2.25 pounds active ingredient per acre. The yield in corn seed was measured at harvest as percent yield relative to unsprayed (WeatherMax 0) MON88017 plots. The results shown in Table 1 demonstrate that MON88017 is highly tolerant to glyphosate and were not reduced in yield, surprisingly the treated plots averaged higher in yield than the treated plots.

[Para 72] Table 1. Percent Yield of MON88017 treated with glyphosate compared to untreated

| [Para 73] Treatment              | Replications |       |       |       |       | Average |
|----------------------------------|--------------|-------|-------|-------|-------|---------|
| WeatherMax 0 - V4                | 100.0        | 100.0 | 100.0 | 100.0 | 100.0 | 100.0   |
| WeatherMax 1.125 + 1.125 - V4/V8 | 99.2         | 118.5 | 102.1 | 100.4 | 107.7 | 105.1   |
| WeatherMax 2.25 + 2.25 - V4/V8   | 99.5         | 118.8 | 107.0 | 97.4  | 115.5 | 105.7   |

[Para 74] Table 1 Legend: Percent yield for each WeatherMax treatment at leaf stages from V4 to V8 were compared to percent yield for WeatherMax treatments at leaf stages from 0 to V4, with results of 0–V4 treatments establishing the 100 percent yield baseline for each treatment repetition.

### [Para 75] EXAMPLE 3

[Para 76] Genomic DNA from MON88017 and control substances was extracted from corn grain by first processing the grain to a fine powder. Approximately 6 grams of the processed grain were transferred to a 50 ml (milliliter) conical tube, then ~16 ml of CTAB extraction buffer [1.5% (w/v) CTAB, 75 mM Tris–HCl pH 8.0, 100 mM EDTA pH 8.0, 1.05 M NaCl, and 0.75% (w/v) PVP (MW 40,000)] and 8 microliter of RNase (10 mg/ml, Roche) were added to the processed grain. The samples were incubated at 65°C for 30–60 minutes with intermittent mixing and then allowed to cool to room temperature. Approximately 15 ml of chloroform:isoamyl alcohol (24:1 (v/v)) was added to the samples. The suspension was mixed for 5 minutes and the two phases separated by centrifugation at ~16,000 x *g* for 5 minutes at room temperature. The aqueous (upper) layer was transferred to a clean 50 ml conical tube. Approximately 1/10 volume (~1.5 ml) of 10% CTAB buffer [10% (w/v) CTAB and 0.7 M NaCl] and an equal volume of chloroform:isoamyl alcohol [24:1 (v/v)] was added to the aqueous phase, which was then mixed for 5 minutes. The samples were centrifuged at ~16,000 x *g* for 5

minutes to separate the phases. The aqueous (upper) layer was removed, mixed with an equal volume (~15 ml) of CTAB precipitation buffer [1% (w/v) CTAB, 50 mM Tris pH 8.0, and 10 mM EDTA pH 8.0] and allowed to stand at room temperature for 1–2 hours. The samples were centrifuged at  $\sim 10,000 \times g$  for 10 minutes at room temperature to pellet the DNA. The supernatant was discarded, and the pellet was dissolved in approximately 2 ml of high salt TE buffer (10mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, and 1 M NaCl). Gentle swirling at 37°C was performed to aid in dissolution of the pellet. If necessary, samples were centrifuged at  $\sim 23,000 \times g$  at room temperature for 2 minutes to pellet and remove debris. Approximately 1/10 volume (~150  $\mu$ l) of 3 M NaOAc (pH 5.2) and 2 volumes (~4 ml relative to the supernatant) of chilled 100% ethanol were added to precipitate the DNA. The precipitated DNA was spooled into a microcentrifuge tube containing 70% ethanol. The DNA was pelleted in a microcentrifuge at maximum speed ( $\sim 14,000$  rpm) for ~5 minutes, vacuum-dried, and re-dissolved in TE buffer (pH 8.0). The DNA was then stored in a 4°C refrigerator.

[Para 77] Ten  $\mu$ g of genomic DNA was digested with a selected restriction enzyme, *e.g.*, Sspl, EcoRV, Scal, and SmaI, in 200  $\mu$ l enzyme restriction buffer at 37°C for more than 4 hours, and then inactivate enzyme at 70–80°C for 15 minutes. DNA was extracted with Phenol and chloroform, and precipitated with EtOH, and dissolved in 200  $\mu$ l. The DNA was then self-ligated in 1 ml ligation buffer and T4 DNA ligase at 4°C for overnight. The ligation reaction was inactivated at 70–80°C for 15 minutes, and precipitated with EtOH, and dissolved in 200  $\mu$ l. The DNA was then used as template for PCR with specific primers within the CP4 EPSPS or CRY3Bb coding regions using High Fidelity Expand System (Roche), following the protocol provided by the Manufacture. A secondary PCR with nested primers were used for specific amplification. The PCR product was then cloned for sequencing analysis. A Universal GenomeWalker kit (cat# K1807–

1, Clontech, Palo Alto, CA) was used for isolation of the 5' and 3' transgene/genomic region DNA using adapter primers AP1 and AP2 included therein and the protocol provided by the manufacture.

**[Para 78]** The 5' (SEQ ID NO:3, Figure 3) and 3' (SEQ ID NO:4, Figure 4) transgene/genomic region DNA are isolated from the MON88017 genomic DNA utilizing PCR. Total genomic DNA (~10 µg) is digested with the restriction enzymes. The QIAquick PCR Purification columns are used to purify the DNA after digesting overnight at 37°C. The DNA is eluted from the columns with 50µl of water and then diluted to 1ml. The diluted eluate (85 µl) is combined with 10 µl of buffer (10X) and 5 µl of T4 Ligase to circularize the fragments. After an overnight incubation at 16°C, the ligase is heat inactivated at 70°C. The samples are amplified by PCR with a series of nested primers. The primer combinations for isolation of the 3' transgene/genome region included SEQ ID NO:8, SEQ ID NO:9, and AP1 as primary primers, SEQ ID NO:10 and AP2 as the secondary primers, and SEQ ID NO:11 for use as a sequencing primer; the isolation of the 5' transgene/genome region included SEQ ID NO:12, SEQ ID NO:14 and AP1 as primary primers, SEQ ID NO:13, SEQ ID NO:15 and AP2 as secondary primers.

**[Para 79]** The conditions for the PCR include: primary PCR = 7 cycles of 94°C for 2 seconds, 72°C for 10 minutes; 37 cycles of 94°C for 2 seconds, 67°C for 10 minutes; 1 cycle of 67°C for 10 minutes; secondary and tertiary PCR = 5 cycles of 94°C for 2 seconds, 72°C for 10 minutes; 24 cycles of 94°C for 2 seconds, 67°C for 10 minutes; 1 cycle of 67°C for 10 minutes.

**[Para 80]** Alternatively, DNA amplification by PCR of the 5' and 3' transgene/genome insert regions of the MON88017 event can be performed with conditions that include: 7 cycles of 94°C for 25 seconds, 72°C for 3 minutes; 37 cycles of 94°C for 25 seconds, 67°C for 3 minutes; 1 cycle of 67°C for 7 minutes. All subsequent

amplifications conducted with the following conditions: 7 cycles of 94°C for 2 seconds, 72°C for 4 minutes; 37 cycles of 94°C for 2 seconds, 67°C for 4 minutes; 1 cycle of 67°C for 7 minutes. All amplicons are visualized on 0.8% agarose gels stained with ethidium bromide. The DNA is prepared for sequencing either by purifying the PCR samples directly with the QIAquick PCR Purification kit (cat# 28104, Qiagen Inc., Valencia, CA) or by extracting the appropriate fragment from the gel and using the QIAquick Gel Extraction kit (cat #28704, Qiagen Inc.). The DNA fragments from the flanking regions of MON88017 transgene/genomic insert were subcloned using a TOPO TA Cloning® kit (Invitrogen, Carlsbad, CA). The DNA sequence of the 5' transgene/genomic region is shown in Figure 3, the DNA sequence of the 3' transgene/genomic region is shown in Figure 4 and the complete transgene insert and linked flanking genomic DNA is shown in Figure 5.

[Para 81] The full-length transgene/genomic insert sequence (SEQ ID NO:5, Figure 5) was isolated from MON88017 genomic DNA by overlapping PCR products. A series of DNA primers were designed to produce amplicons that contain DNA fragments of the transgene insert and a portion of the adjacent flanking genomic regions from the MON88017 genome. The DNA fragments were sequenced, the sequences were combined to create a contig of the fragment sequences that is SEQ ID NO:5 of the present invention. The DNA primer pair combinations were: SEQ ID NO:16 and SEQ ID NO:17, SEQ ID NO:18 and SEQ ID NO:17, SEQ ID NO:19 and SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:24, SEQ ID NO:25 and SEQ ID NO:26, SEQ ID NO:25 and SEQ ID NO:27. Total genomic DNA was used for all PCR reactions. All amplicons were visualized on 0.8% agarose gels stained with ethidium bromide. The DNA was prepared for sequencing either by purifying the PCR samples directly with the QIAquick PCR Purification kit or by extracting the appropriate fragment from the gel and using the

QIAquick Gel Extraction kit. The DNA sequence was produced using DNA sequence analysis equipment (ABI Prism™ 377, PE Biosystems, Foster City, CA) and DNASTAR sequence analysis software (DNASTAR Inc., Madison, WI).

**[Para 82] EXAMPLE 4**

**[Para 83]** DNA event primer pairs are used to produce an amplicon diagnostic for corn event MON88017. An amplicon diagnostic for MON88017 comprise at least one junction sequence, SEQ ID NO:1 or SEQ ID NO:2. Event primer pairs that will produce a diagnostic amplicon for MON88017, in which the primer pairs include, but are not limited to SEQ ID NO:6 and SEQ ID NO:7 for the 5' amplicon sequence as outlined in Table 2. The location of primer SEQ ID NO:6 is in the corn genome as shown in Figure 3 beginning at nucleotide position 952. The location of primer SEQ ID NO:7 is in the transgene insert as shown in Figure 5 beginning at nucleotide position 450. The expected amplicon size using SEQ ID NO:6 and SEQ ID NO:7 in a DNA amplification method with MON88017 DNA is approximately 550 bps. In addition to these primer pairs, any primer pair derived from SEQ ID NO:3 or SEQ ID NO:4 that in a DNA amplification reaction produces an amplicon diagnostic for MON88017 or progeny thereof is an aspect of the present invention. Any single isolated DNA polynucleotide primer molecule comprising at least 11 contiguous nucleotides of SEQ ID NO:3, or its complement that is useful in a DNA amplification method to produce an amplicon diagnostic for MON88017 is an aspect of the invention. Any single isolated DNA polynucleotide primer molecule comprising at least 11 contiguous nucleotides of SEQ ID NO:4, or its complement that is useful in a DNA amplification method to produce an amplicon diagnostic for MON88017 is an aspect of the invention. An example of the amplification conditions for this analysis is illustrated in Table 2 and Table 3, however, any

modification of these methods or the use DNA primers homologous or complementary to SEQ ID NO:3 or SEQ ID NO:4 or DNA sequences of the genetic elements contained in the transgene insert (SEQ ID NO:5) of MON88017 that produce an amplicon diagnostic for MON88017, is within the art. A diagnostic amplicon comprises a DNA molecule homologous or complementary to at least one transgene/genomic junction DNA (SEQ ID NO:1 or SEQ ID NO:2) or a substantial portion thereof.

**[Para 84]** An analysis for event MON88017 plant tissue sample should include a positive tissue control from event MON88017, a negative control from a corn plant that is not event MON88017, and a negative control that contains no corn genomic DNA. A primer pair that will amplify an endogenous corn DNA molecule will serve as an internal control for the DNA amplification conditions, an example of these are SEQ ID NO:28 and SEQ ID NO:29 that amplifies an approximately 239 bp DNA fragment. Additional primer sequences can be selected from SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5 by those skilled in the art of DNA amplification methods, and conditions selected for the production of an amplicon by the methods shown in Table 2 and Table 3 may differ, but result in an amplicon diagnostic for event MON88017 DNA. The use of these DNA primer sequences with modifications to the methods of Table 2 and 3 are within the scope of the invention. The amplicon produced by at least one DNA primer sequence derived from SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5 that is diagnostic for MON88017 is an aspect of the invention.

**[Para 85]** DNA detection kits that contain at least one DNA primer derived from SEQ ID NO:3 or SEQ ID NO:4, or SEQ ID NO:5 that when used in a DNA amplification method produces a diagnostic amplicon for MON88017 is an aspect of the invention. A corn plant or seed, wherein its genome will produce an amplicon diagnostic for MON88017 when tested in a DNA amplification method is an aspect of the invention. The assay for



the MON88017 amplicon can be performed by using a Stratagene Robocycler, MJ Engine, Perkin-Elmer 9700, or Eppendorf Mastercycler Gradient thermocycler as shown in Table 3, or by methods and apparatus known to those skilled in the art.

[Para 86] Table 2. PCR procedure and reaction mixture conditions for the identification of MON88017 the 5' transgene insert/genomic junction region.

| Step | Reagent  | Amount  | Comments   |
|------|--|---|--|
| 1    | Nuclease-free water  | add to final volume of 20 $\mu$ l   | -  |
| 2    | 10X reaction buffer (with $MgCl_2$ )   | 2.0 $\mu$ l   | 1X final concentration of buffer, 1.5 mM final concentration of $MgCl_2$ |
| 3    | 10 mM solution of dATP, dCTP, dGTP, and dTTP   | 0.4 $\mu$ l   | 200 $\mu$ M final concentration of each dNTP                             |
| 4    | event primer 1 (SEQ ID NO:6) (resuspended in 1X TE buffer or nuclease-free water to a concentration of 10 $\mu$ M)   | 0.4 $\mu$ l   | 0.2 $\mu$ M final concentration  |
| 5    | event primer 2 (SEQ ID NO:7) (resuspended in 1X TE buffer or nuclease-free water to a concentration of 10 $\mu$ M)   | 0.4 $\mu$ l   | 0.2 $\mu$ M final concentration  |
| 6    | RNase, DNase free (500 ng/ $\mu$ l)  | 0.1 $\mu$ l   | 50 ng/reaction   |
| 7    | <i>REDTaq</i> DNA polymerase (1 unit/ $\mu$ l)   | 1.0 $\mu$ l (recommended to switch pipets prior to next step)   | 1 unit/reaction  |
| 8    | Extracted DNA (template): <ul style="list-style-type: none"> <li>• Samples to be analyzed               <ul style="list-style-type: none"> <li>* individual leaves</li> <li>* pooled leaves (maximum of 20 leaves/pool)</li> </ul> </li> <li>• Negative control</li> <li>• Negative control</li> <li>• Positive control</li> </ul> | <ul style="list-style-type: none"> <li>• 10-200 ng of genomic DNA</li> <li>• 200 ng of genomic DNA</li> <li>• 50 ng of corn genomic DNA (not MON88017)</li> <li>• no template DNA</li> <li>• 50 ng of MON88017 genomic DNA</li> </ul> | -  |
| 9    | Gently mix and add 1-2 drops of mineral oil on top of each reaction.   |   |  |

[Para 87] Table 3. Suggested PCR parameters for various commercially available thermocyclers.

| Cycle No. | Settings: Stratagene Robocycler                   |
|-----------|---|
| 1         | 94°C 3 minutes                                    |
| 38        | 94°C 30 seconds<br>59°C 1 minute<br>72°C 1 minute |
| 1         | 72°C 10 minutes                                   |

| Cycle No. | Settings: MJ Engine, Perkin-Elmer 9700 or Eppendorf Mastercycler Gradient |
|-----------|---|
| 1         | 94°C 3 minutes  |
| 38        | 94°C 15 seconds<br>59°C 30 seconds<br>72°C 1 minute                       |
| 1         | 72°C 10 minutes   |

[Para 88] Proceed with the DNA amplification in a Stratagene Robocycler, MJ Engine, Perkin-Elmer 9700, or Eppendorf Mastercycler Gradient thermocycler using the following cycling parameters. The MJ Engine or Eppendorf Mastercycler Gradient thermocycler should be run in the calculated mode. Run the Perkin-Elmer 9700 thermocycler with the ramp speed set at maximum.

[Para 89] Example 5

[Para 90] Southern blot analysis was performed on genomic DNA was isolated from the MON88017 and control corn tissue as described in Example 3. Quantitation of DNA samples was performed using a Hoefer DyNA Quant 200 Fluorometer (Pharmacia, Uppsala, Sweden) with Roche molecular size marker IX as a DNA calibration standard.

[Para 91] Approximately 20 µg of genomic DNA from the test substance and 10 µg of genomic DNA from the control substance were used for restriction enzyme digestions. For the insert stability analysis, approximately 10 µg of genomic DNA from the test substance were used. Overnight digests were performed at 37°C in a total volume of ~500 µl using 1.00 units of the appropriate restriction enzyme. After digestion, the samples were precipitated by adding 1/10 volume (50 µl) of 3 M NaOAc (pH 5.2) and 2 volumes (1 ml relative to the original digest volume) of 100% ethanol, followed by incubation in a -20°C freezer for at least 30 minutes. The digested DNA was pelleted at maximum speed in a microcentrifuge, washed with 70% ethanol, dried, and re-dissolved in TE buffer.

[Para 92] DNA probes were prepared by PCR amplification of plant expression cassette portion of pMON53616 template DNA. Approximately 25 ng of each probe (except the NOS 3' and TaHsp17 3' polyadenylation sequences) were labeled with <sup>32</sup>P-dCTP (6000 Ci/mmol) by a random priming method (RadPrime DNA Labeling System, Life Technologies). The NOS 3' and tahsp17 3' polyadenylation sequences were labeled by PCR using 25 ng of DNA probe template in the following manner: sense and antisense primers specific to the template (0.25 mM each); 1.5 mM MgCl<sub>2</sub>; 3 mM each of dATP, dGTP and dTTP; ~100 mCi of <sup>32</sup>P-dCTP (6000 Ci/mmol); and 2.5 Units of Taq DNA polymerase in a final volume of 20 µl. The cycling conditions were as follows: 1 cycle at 94°C for 3 minutes; 2 cycles at 94°C for 45 seconds, 52°C for 30 seconds, 72°C for 2 minutes; and 1 cycle at 72°C for 10 minutes. All radiolabeled probes were purified using a Sephadex G-50 column (Roche, Indianapolis, IN).

[Para 93] Synthetic DNA molecules for use as probes for marker assisted breeding methods or for the detection of the MON88017 DNA in a sample can be made comprising the DNA sequence of

the transgene/genome junction DNA molecule described in SEQ ID NO:1 and SEQ ID NO:2 or a substantial portion thereof.

**[Para 94] EXAMPLE 6**

**[Para 95]** The methods used to identify heterozygous from homozygous progeny containing event MON88017 are described in a zygosity assay for which examples of conditions are described in Table 4 and Table 5. The DNA primers used in the zygosity assay are primers (SEQ ID NO:30), (SEQ ID NO:31), (SEQ ID NO:32), 6FAM™ labeled primer (SEQ ID NO:33) and VIC™ labeled primer (SEQ ID NO:34), 6FAM and VIC are florescent dye products of Applied Biosystems (Foster City, CA) attached to the DNA primer.

**[Para 96]** SEQ ID NO:30, SEQ ID NO:31 and SEQ ID NO:32 when used in these reaction methods produce a DNA amplicon for non-transgenic corn, two DNA amplicons for heterozygous corn containing event MON88017 DNA, and a DNA amplicon for homozygous MON88017 corn that is distinct from any other non-MON88017 corn plant. The controls for this analysis should include a positive control from homozygous and heterozygous corn containing event MON88017 DNA, a negative control from non-transgenic corn, and a negative control that contains no template DNA. This assay is optimized for use with a Stratagene Robocycler, MJ Engine, Perkin-Elmer 9700, or Eppendorf Mastercycler Gradient thermocycler. Other methods and apparatus known to those skilled in the art that produce amplicons that identify the zygosity of the progeny of crosses made with MON88017 plants is within the skill of the art.

[Para 97] Table 4. Zygosity assay reaction solutions

| Step | Reagent  | Amount   | Comments                         |
|------|--|--|----------------------------------|
| 1    | Nuclease-free water  | add to 10 $\mu$ l final volume   | -                                |
| 2    | 2X Universal Master Mix (Applied Biosystems cat. # 4304437)  | 5 $\mu$ l  | 1 X final concentration          |
| 3    | Primers SEQ ID NO:30, 31, and 32 (resuspended in nuclease-free water to a concentration of 20 $\mu$ M)   | 0.5 $\mu$ l  | 0.25 $\mu$ M final concentration |
| 4    | Primer 6FAM <sup>TM</sup> (resuspended in nuclease-free water to a concentration of 10 $\mu$ M)  | 0.2 $\mu$ l  | 0.4 $\mu$ M final concentration  |
| 5    | Primer VIC <sup>TM</sup> (resuspended in nuclease-free water to a concentration of 10 $\mu$ M)   | 0.2 $\mu$ l  | 0.15 $\mu$ M final concentration |
| 6    | REDTaq DNA polymerase (1 unit/ $\mu$ l)  | 1.0 $\mu$ l (recommended to switch pipets prior to next step)  | 1 unit/reaction                  |
| 7    | Extracted DNA (template): <ul style="list-style-type: none"> <li>• Samples to be analyzed (individual leaves)</li> <li>• Negative control</li> <li>• Negative control</li> <li>• Positive control</li> <li>• Positive control</li> </ul> | 3.0 $\mu$ l <ul style="list-style-type: none"> <li>• 4-80 ng of genomic DNA</li> <li>• 4 ng of non-transgenic corn genomic DNA</li> <li>• no DNA template (solution in which DNA was resuspended)</li> <li>• 4 ng of genomic DNA from known event MON88017 heterozygous corn</li> <li>• 4 ng of genomic DNA from known event MON88017 homozygous corn</li> </ul> | Diluted in water                 |
| 8    | Gently mix, add 1-2 drops of mineral oil on top of each reaction.  |  |                                  |

**[Para 98] Table 5. Zygosity assay thermocycler conditions**

| <b>Cycle No.</b> | <b>Settings: Stratagene Robocycler</b>                         |
|------------------|--|
| 1                | 94°C 3 minutes   |
| 38               | 94°C 1 minute<br>60°C 1 minute<br>72°C 1 minute and 30 seconds |
| 1                | 72°C 10 minutes  |

| <b>Cycle No.</b> | <b>Settings: MJ Engine or Perkin-Elmer 9700</b>                    |
|------------------|--|
| 1                | 94°C 3 minutes   |
| 38               | 94°C 30 seconds<br>60°C 30 seconds<br>72°C 1 minute and 30 seconds |
| 1                | 72°C 10 minutes  |

| <b>Cycle No.</b> | <b>Settings: Eppendorf Mastercycler Gradient</b>                   |
|------------------|--|
| 1                | 94°C 3 minutes   |
| 38               | 94°C 15 seconds<br>60°C 15 seconds<br>72°C 1 minute and 30 seconds |
| 1                | 72°C 10 minutes  |

**[Para 99]** Proceed with the DNA amplification in a Stratagene Robocycler, MJ Engine, Perkin-Elmer 9700, or Eppendorf Mastercycler Gradient thermocycler using the following cycling parameters. When running the PCR in the Eppendorf Mastercycler Gradient or MJ Engine, the thermocycler should be run in the calculated mode. When running the PCR in the Perkin-Elmer 9700, run the thermocycler with the ramp speed set at maximum.

**[Para 100]** A deposit of corn MON88017 seed disclosed above and recited in the claims, has been made under the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110. The ATCC accession number is PTA-5582. The deposit will be maintained in the depository for a period of 30 years, or 5 years after the last

request, or for the effective life of the patent, whichever is longer, and will be replaced as necessary during that period.

**[Para 101] Example 7**

**[Para 102]** Products such as foodstuffs and commodities can be produced from corn event MON88017, and such foodstuffs and commodities are expected to contain nucleotide sequences that, if detected in sufficient levels in such foodstuffs and commodities, can be diagnostic for the presence of corn event MON88017 materials within such commodities and foodstuffs. Examples of such foodstuffs and commodities include but are not limited to corn oil, corn meal, corn flour, corn gluten, corn cakes, corn starch, and any other foodstuff intended for consumption as a food source by an animal or otherwise, intended as a bulking agent, or intended as a component in a makeup composition for cosmetic use, etc. A nucleic acid detection method and/or kit based on a probe or a primer pair wherein the probe sequence or the sequence of the primers are selected from the group of sequences as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5 may be developed that allows the detection of a MON88017 nucleotide sequence such as SEQ ID NO:1 or SEQ ID NO:2 in a biological sample, and such detection would be diagnostic for the corn event MON88017 in such sample.

**[Para 103]** Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications that are within the spirit and scope of the appended claims.

**[Para 104]** All publications and published patent documents cited in this specification are incorporated herein by reference to the same extent as if each individual publication or patent application



was specifically and individually indicated to be incorporated by reference.